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# Antimycobacterial potential of the juniper berry essential oil in tap water

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*Mycobacterium avium* complex-related diseases are often associated with poorly maintained hot water systems. This calls for the development of new control strategies. The aim of this study was to investigate the activity of essential oils (EOs) from the Mediterranean plants, common juniper, immortelle, sage, lavandin, laurel, and white cedar against *Mycobacterium avium* ssp. *avium*, *Mycobacterium intracellulare*, and *Mycobacterium gordonae* in culturing broth and freshwater as their most common habitat. To do that, we developed a new method of water microdilution to determine their minimal effective concentrations (MEC). The most active EO was the one from the common juniper with the MEC of 1.6 mg $mL^{-1}$. Gas chromatography / mass spectrometry the juniper EO identified monoterpenes (70.54 %) and sesquiterpenes (25.9 %) as dominant component groups. The main monoterpene hydrocarbons were α-pinene, sabinene, and β-pinene. The juniper EO significantly reduced the cell viability of *M. intracellulare* and *M. gordonae* at MEC, and of *M. avium* at 2xMEC. Microscopic analysis confirmed its inhibitory effect by revealing significant morphological changes in the cell membrane and cytoplasm of all three bacteria. The mode of action of the juniper EO on the cell membrane was confirmed by a marked leakage of intracellular material. Juniper EO has a great practical potential as a complementary or alternative water disinfectant in hot water systems such as baths, swimming pools, spa pools, hot tubs, or even foot baths/whirlpools.

KEY WORDS: Helichrysum italicum; Juniperus communis; Lavandula hybrida; Laurus nobilis; *minimum effective concentration;* Mycobacterium avium; Mycobacterium gordonae; Mycobacterium intracellulare; *nontuberculous mycobacteria;* Salvia officinalis; *tap water;* Thuja occidentalis; *water infections; water microdilution*

Nontuberculous mycobacteria (NTM) are a heterogeneous group of environmental bacteria, most commonly isolated from water, soil, dust, and animals. Some species may be pathogenic to humans, especially the immunocompromised ones, and can cause pulmonary and skin infections (1). Humans are most often exposed to NTM through freshwater (2-5). In the aquatic environment, NTM form biofilms to resist standard decontamination and disinfection (2, 3, 6, 7). The growing number of resistant microorganisms calls for new methods, and plants offer promising antimicrobial activity (8), their essential oils (EOs) in particular.

EOs are volatile, natural, complex compounds, produced by aromatic plants as secondary metabolites. Hence their strong aroma (9). They mainly consist of terpenoids and terpenes (especially hemiterpenes, monoterpenes, sesquiterpenes, and diterpenes), and a variety of aromatic and aliphatic compounds (acids, alcohols, aldehydes, acyclic esters, or lactons) (9-11). Their antimicrobial activity depends on their chemical composition, functional groups, and synergism between components, if there is one (9, 10, 12). Even EOs of the same plant differ in composition due to a number of factors such as geographical location, plant maturity and age, part of the plant used, and distillation method. A number of studies have already investigated the antimicrobial effects of EOs against NTM in a variety of matrices, but none that we know of used tap water as the matrix, even though it is their most common natural environment and source of infection as well, in which they develop higher resistance than in artificial (laboratory) environments (9, 13). Our aim was, therefore, to investigate the antimycobacterial effectiveness of EOs from Mediterranean plants in tap water. To do that, we developed a new method of water microdilution to determine their minimal effective concentrations (MEC).

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## MATERIALS AND METHODS

### *Essential oils, gas chromatography, and compounds*

For the experiments we used natural commercial EOs distilled from the wild common juniper (*Juniperus*

*communis*), immortelle (*Helichrysum italicum*), sage (*Salvia officinalis*), lavandin (*Lavandula hybrida*), laurel (*Laurus nobilis*), and white cedar (*Thuja occidentalis*) growing on the Croatian coast. The oils were purchased from IREKS AROMA, Zagreb, Croatia. The most common organic compound of the terpene class in the EOs, α-pinene, was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Having determined the most efficient oil, we then analysed it for components with an Agilent gas chromatograph/mass spectrometer (GC-MS) model 7820A equipped with a mass selective detector (MSD) model 5977E and a HP-5MS column (5 % phenyl-methylpolysiloxane) (Agilent Technologies, Palo Alto, CA, USA). The GC conditions were similar to those described previously (14). In brief, the oven temperature started at 70 °C for 2 min, then ramped from 70 to 200 °C at 3 °C $min^{-1}$, and remained isothermal at 200 °C for 15 min. The carrier gas was helium (flow rate: 1.0 mL $min^{-1}$). The MSD (EI mode) was operated at 70 eV, and the range was 30-300 atomic mass units (amu), as reported earlier (14, 15). Individual compound peaks were identified by comparing their retention indices (relative to $C_9$-$C_{25}$ *n*-alkanes) with those of the available authentic samples and literature data (16) and by comparing their mass spectra with the Wiley 09 MS library (Wiley, New York, NY, USA) and NIST14 (D-Gaithersburg) database. The percentage of the composition was calculated from the GC peak using the normalisation method (without correction factors).

*Tap water sampling*

For the experiments with tap water we used water from the public water supply of the city of Rijeka with the following properties: salinity 0, pH 7.5-8.0, and conductivity 216-300 µS $cm^{-1}$. In other words, it was medium hard water. We poured the water in a glass bottle and left it at room temperature to dechlorinate for two days. Then we sterilised it by autoclaving at 121 °C for 15 min, cooled to room temperature, and stored at 4 °C until use.

*Bacterial cultures*

For the experiments we used the following bacterial strains: *Mycobacterium avium* ssp. *avium* (serotype 2) ATCC 25291 (*M. avium*), *Mycobacterium intracellulare* ATCC 13950 (*M. intracellulare*), and *Mycobacterium gordonae* ATCC 14470 (*M. gordonae*). The strains were cultivated twice in Middlebrook 7H9 broth (7H9, Difco, Detroit, Michigan, USA) with 10 % albumin-dextrose-catalase (ADC, Biolife Italiana, Milano, Italy) and 0.05 % Tween 80 (Tw80, Biolife Italiana, Milano, Italy) at 30 °C (*M. gordonae*) or 37 °C (*M. avium* and *M. intracellulare*) for two weeks to obtain $10^8$ CFU $mL^{-1}$. The bacteria were then frozen at -80 °C with 10 % glycerol. For each experiment, an aliquot was thawed and cultured in 7H9 for two weeks and then the culture was incubated on Middlebrook 7H10 agar (7H10, Difco) with 10 % oleic acid-albumin-dextrose-catalase (OADC, Biolife Italiana) and 0.05 % Tw80 at 30 °C (*M. gordonae*) or 37 °C (*M. avium* and *M. intracellulare*) for two more weeks. The initial inocula were later verified by diluting and plating the culture onto 7H10 with 10 % OADC and 0.05 % Tw80 and by incubating them at 30 °C (*M. gordonae*) or 37 °C (*M. avium* and *M. intracellulare*) for four to six weeks before the colonies were counted (17).

*Determination of minimum inhibitory concentration and minimum bactericidal concentration in broth*

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the tested EO samples were determined using a broth microdilution method, as follows: EO was double diluted in 7H9 with 10 % OADC and 0.05 % Tw80, starting from 0.1 to the final 51.2 mg $mL^{-1}$ in a sterile 96-well microtitre plate (Vacutest Kima s.r.l., Arzergrande, Italy). The final concentration of the solvent dimethyl sulphoxide (DMSO) was about 10 %. Each dilution was mixed with a mycobacterial suspension ($1x10^6$ CFU $mL^{-1}$ per well) and resazurin (0.015 % solution) (Sigma-Aldrich) to reach the final volume of 200 µL. Resazurin is an oxidation-reduction indicator used for the evaluation of cell growth. It is a blue non-fluorescent and non-toxic dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases in viable cells (18). In addition to negative sterile control (without bacteria), we also used a mixture with amikacin for positive control. The plates were incubated at 30 °C (*M. gordonae*) or 37 °C (*M. avium* and *M. intracellulare*) and swirled at 120 rpm with a Heidolph UNIMAX 1010 shaker (Schwabach, Germany) for 24 h. MIC was the lowest concentration without change in colour. MBC was determined by inoculating the dilutions that showed no colour change with *M. gordonae* or *M. avium* or *M. intracellulare* on 7H10 supplemented with 10 % OADC and by incubating them at 30 °C (*M. gordonae*) or 37 °C (*M. avium* and *M. intracellulare*) for another four weeks. MBC was the lowest concentration of EO that killed ≥99 % of the bacteria. The concentrations are expressed in mg $mL^{-1}$ (18).

*Determination of minimal effective concentration in tap water*

To determine the minimal effective concentration (MEC) in sterile tap water, we repeated the same procedure as above, but instead of 7H9 with 10 % OADC and 0.05 % Tw80 broth we only used sterile tap water mixed with 0.05 % Tw80. MEC was the lowest concentration of EO that killed ≥99 % of the bacteria. The concentration is expressed in mg $mL^{-1}$.

*Determining mycobacterial survival in sterile tap water*

This method allows the characterisation of the antibacterial activity of an EO over time. We prepared a

suspension of $10^8$ CFU mL$^{-1}$ of each mycobacterial strain in sterile tap water with 0.05 % Tw80 and then added half or the entire MEC of the EO and incubated the mix by stirring it at 30 °C (*M. gordonae*) or 37 °C (*M. avium* and *M. intracellulare*). On days 0, 1, and 2, we removed 100 µL of the suspension and determined bacterial CFU mL$^{-1}$. For growth control, we used unexposed bacteria (19).

*Determining bacteriolysis*

This method determines if there is a bacteriolytic action by measuring the absorbance at 620 nm, as non-lysed bacteria absorb at 620 nm. If there is a bacteriolysis, the absorbance at 620 nm will decrease over time. Bacterial suspension ($10^8$ CFU mL$^{-1}$) was placed in a sterile tube in the absence (negative control) or in the presence of EO at two concentrations: one MEC and the other two times the MEC. The obtained suspensions were then incubated with agitation for 24 h, after which we measured the absorbance at 620 nm. For the blank we used sterile tap water with 0.05 % Tw80 and EO at MEC or 2xMEC. The results are expressed as the relative optical density ($OD_{620}$) (20).

*Leakage of cellular metabolites*

Two-week bacterial cultures in 7H9 with 10 % ADC and 0.05 % Tw80 were transferred into sterile centrifuge tubes and centrifuged at 3500 *g* for 10 min. The supernatant was discarded and the pellet resuspended in sterile tap water. The suspension was centrifuged and resuspended twice in sterile tap water with 0.05 % Tw80. $OD_{600}$ of each sample was adjusted to around 1.0, which corresponds to $10^8$ CFU mL$^{-1}$, and the bacterial counts verified by diluting and plating ten-fold serial dilutions. Aliquots of each bacterial suspension were then placed in sterile flasks and mixed with the EO at MEC or 2xMEC. Flasks containing only bacterial cultures served as controls. After a 24-hour incubation at 30 °C (*M. gordonae*) or 37 °C (*M. avium* and *M. intracellulare*), the suspensions were centrifuged at 3500 *g* for 10 min. The supernatants were used to quantitate DNA at absorbance of 260 nm (A260) and proteins at 280 nm (A280) (21, 22).

*Determining the EO DPPH radical scavenging activity (RSA)*

The radical scavenging activity (RSA) of the EO was determined by measuring 2,2-diphenyl-1-picrylhydrazyl (DPPH·) inhibition (23). We added the EO in MEC to a freshly prepared, 0.1 mmol L$^{-1}$ DPPH· solution in ethyl acetate in order to obtain a 4 % w/v EO solution. After 10 s of vigorously mixing on a vortex agitator, the absorbance of the mixture was measured spectrophotometrically (Cary 100 Bio WINUV, Mulgrave, Australia) at 515 nm every minute over 60 min until the reaction reached a steady state. The total RSA of the EO was expressed as the percentage of DPPH left after 60 min, as follows:

$$\text{DPPH}^{\cdot}\ \text{scavenging}\ (\%) = \frac{A_0 - A_{sample}}{A_0} \cdot 100$$

where $A_0$ is the absorbance of the ethyl acetate DPPH· solution without the sample as negative control and $A_{sample}$ the absorbance of the mix at t=60 min.

DPPH inhibition was compared with the standard curve of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Trolox, Sigma-Aldrich Chemie, Germany) in the range of 0-0.21 mmol L$^{-1}$. In quantitative terms, radical scavenging was expressed as mmoles of Trolox equivalents per kg of EO (mmol TEAC kg$^{-1}$EO).

*Transmission electron microscopy*

To evaluate structural changes, we analysed the morphologies of the bacteria exposed to the selected EO. Briefly, *M. avium* was grown on 7H10 with 10 % OADC and 0.05 % Tw80 for four to six weeks. Ten microliters of the bacterial suspension ($10^8$ CFU mL$^{-1}$) was placed on Formwar-coated copper grids (Agar Scientific Ltd, Essex, United Kingdom) for 2 min. After that, the excess of liquid was wicked off the grids with Watman no. 3 filter paper. The bacteria remaining on the grids were stained with 1 % phosphotungstic acid (PTA; Sigma-Aldrich) for 1 min, and the excess of PTA carefully removed with filter paper. The grids were then left to dry on air for a few minutes. The bacteria were inspected on a transmission electron microscope (JEM-2100F, Jeol, Japan).

*Statistical analysis*

The results were analysed with the STATISTICA 12. 0 (StatSoft, Tulsa, OK, USA) and expressed as means ± standard deviation (SD). The Kolmogorov-Smirnov test confirmed normal distribution. Differences between the groups of samples were analysed with the Kruskal-Wallis test by ranks, while the effects of EO on the mycobacteria were tested with the Mann-Whitney U test. Differences with $p<0.05$ were considered statistically significant.

## RESULTS

The most effective EO tested against the selected mycobacteria in this study was that of the common juniper, with the MIC/MBC in broth and MEC in sterile tap water of 1.6 mg mL$^{-1}$. Table 1 shows the efficiency of all tested EOs in both media.

Spectrometry of the juniper EO identified 36 components, which made 96.44 % of the oil. The main components were monoterpenes (70.54 %) and sesquiterpenes (25.9 %) (Table 2).

Figure 1 shows the inhibition of the mycobacteria with the juniper EO at half the MEC and full MEC.

Figure 2 shows the bacteriolytic action of the juniper EO at MEC and double the MEC over 24 h, while Figures 3 and 4 show the protein (280 nm) and DNA (260 nm)

**Table 1** *Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum effective concentration (MEC) against three mycobacterial strains*

| Essential oil | MIC / MBC in 7H9 broth (mg mL$^{-1}$) | | | MEC in sterilised tap water (mg mL$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | *M. avium* | *M. intracellulare* | *M. gordonae* | *M. avium* | *M. intracellulare* | *M. gordonae* |
| *Thuja occidentalis* | 6.4 / 6.4 | 6.4 / 6.4 | 12.8 / 12.8 | >51.2 | >51.2 | >51.2 |
| *Juniperus communis* | 1.6 / 1.6 | 1.6 / 1.6 | 1.6 / 1.6 | 1.6 | 1.6 | 1.6 |
| *Helichrysum italicum* | 3.2 / 3.2 | 3.2 / 3.2 | 3.2 / 3.2 | 3.2 | 3.2 | 1.6 |
| *Laurus nobilis* | 12.8 / 6.4 | 12.8 / 6.4 | 3.2 / 1.6 | 25.6 | 25.6 | 25.6 |
| *Lavandula hybrida* | 3.2 / 1.6 | 3.2 / 1.6 | 6.4 / 3.2 | 12.8 | 12.8 | 25.6 |
| *Salvia officinalis* | 12.8 / 12.8 | 25.6 / 12.8 | 6.4 / 1.6 | 12.8 | 25.6 | 25.6 |
| α-pinene | 12.8 / 12.8 | 12.8 / 12.8 | 6.4 / 1.6 | 12.8 | 12.8 | 12.8 |
| Amikacin | 0.004 / 0.008 | 0.004 / 0.008 | 0.008 / 0.008 | ND | ND | ND |

*ND - not determined*

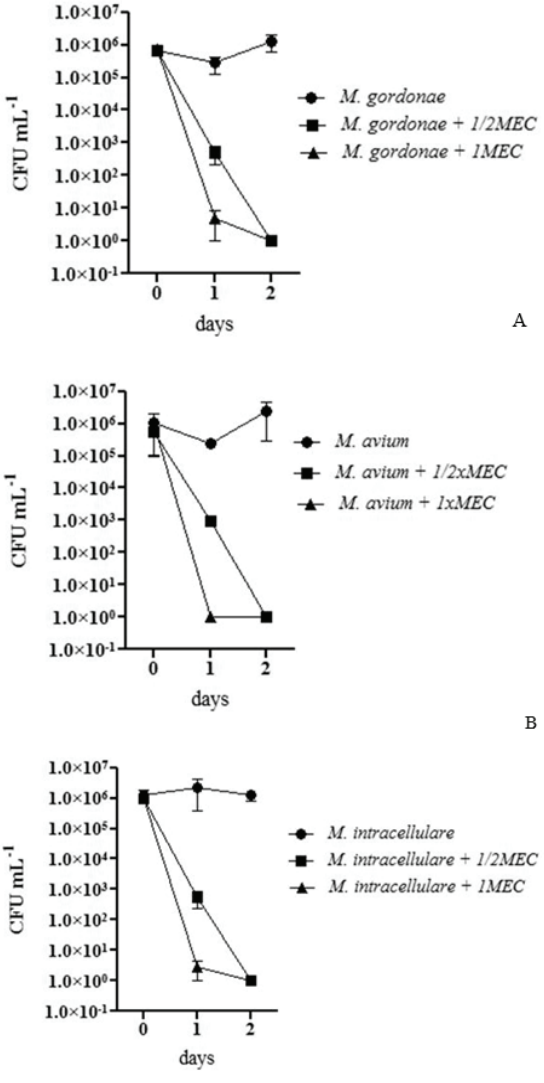


**Figure 1** *Time-kill curves of* M. avium *(A),* M. intracellulare *(B), and* M. gordonae *(C) in sterile tap water in control suspensions (●) and after treatment with juniper EO at half the MEC (■) and MEC (▲)*

leakage due to cell membrane damage at the same EO concentrations. Only *M. avium* did not show a significant leakage at MEC. Morphological changes were clear in the cells of all three bacteria exposed to the juniper EO; cell cytoplasm was destroyed and the cells were clustered, forming small buds on the cell wall (Figure 5B).

As for the DPPH radical-scavenging activity, the juniper EO MEC resulted in a 21.9 % and 26.3 % DPPH inhibition after 30 and 60 min, respectively. The 60-minute inhibition corresponded to 0.0811 mmol kg$^{-1}$ TEAC.

## DISCUSSION

Some authors have shown that the common juniper EO content varies with the geographical origin of the plant, maturity of the berries, age of the plant, meteorological and other microclimatic conditions (e. g. temperature), as well as harvesting and distillation methods (24, 25). Our juniper EO was dominated by α-pinene, which is in agreement with reports from Croatia (16.9–29.17 %) (26, 27), Italy (6.41–52.91 %) (28), Greece (41.3 %) (29), Bulgaria (51.4 %) (30), Macedonia (15.59–43.19 %) (24), Kosovo (23–36.2 %) (31, 32), Serbia (36.6–40.5 %) (33), and Estonia (54.6 %) (34). Monoterpene hydrocarbons, to which α-pinene belongs, can easily pass through the cell membrane lipid bilayer, most probably by diffusion (35). Sikkema et al. (36) reported that cyclic hydrocarbons swell the cell membrane bilayer and increase its permeability in *Escherichia coli*. This permeability is associated with the loss of ions and therefore a drop in membrane potential, collapse of the proton pump, and depletion of the ATP pool. Once the EO has entered the cell, it can coagulate the cytoplasm and damage lipids and proteins. As the cell wall and membrane are already damaged, the cell breaks and macromolecules leak out (8, 35-38). Bakkali et al. (9) also reported much greater sensitivity of the dividing cells, probably because EO penetrated more efficiently at the budding sites. Our morphological analysis has confirmed

**Table 2** *Chemical composition of the juniper EO determined with gas chromatography / mass spectrometry*

| No. | Compound name and class | RI | Area (%) |
|---|---|---|---|
| **Monoterpene hydrocarbons** | | | **66.29** |
| 1. | *α*-Thujene | 933 | 1.94 |
| 2. | *α*-Pinene | 944 | 31.13 |
| 3. | Camphene | 957 | 0.51 |
| 4. | Verbene | 962 | 0.14 |
| 5. | Sabinene | 980 | 9.95 |
| 6. | *β*-Pinene | 983 | 8.50 |
| 7. | *β*-Myrcene | 994 | 4.80 |
| 8. | *α*-Terpinene | 1021 | 0.44 |
| 9. | *p*-Cymene | 1030 | 2.45 |
| 10. | Limonene | 1034 | 4.53 |
| 11. | *γ*-Terpinene | 1064 | 0.88 |
| 12. | *α*-Teroinolene | 1091 | 1.02 |
| **Monoterpene alcohols** | | | **3.81** |
| 13. | Linalool | 1101 | 0.31 |
| 14. | *trans*-Pinocarveol* | 1141 | 0.22 |
| 15. | 4-Terpineol | 1182 | 2.76 |
| 16. | *p*-cymen-8-ol | 1189 | 0.19 |
| 17. | *α*-Terpineol | 1193 | 0.33 |
| **Monoterpene esters** | | | **0.44** |
| 18. | *α*-Fenchyl acetate | 1288 | 0.44 |
| **Sesquiterpene hydrocarbons** | | | **24.86** |
| 19. | *α*-Cubebene | 1353 | 1.34 |
| 20. | *β*-Elemene | 1393 | 2.34 |
| 21. | Isoledene | 1402 | 0.25 |
| 22. | *trans*- *β*-Caryophyllene | 1421 | 2.95 |
| 23. | *γ*-Elemene | 1436 | 0.52 |
| 24. | *α*-Humulene | 1456 | 2.44 |
| 25. | *trans*-*β*-Fernesene | 1460 | 0.44 |
| 26. | *α*-Amorphene | 1479 | 1.09 |
| 27. | Germacrene-D | 1483 | 4.21 |
| 28. | *β*-Selinene | 1487 | 0.75 |
| 29. | *α*-Selinene | 1495 | 1.09 |
| 30. | *α*-Muurolene | 1501 | 0.77 |
| 31. | *δ*-Cadinene | 1526 | 3.33 |
| 32. | *α*-Cadinene | 1540 | 0.18 |
| 33. | Garmacrene-B | 1558 | 2.83 |
| 34. | T-Muurolol | 1644 | 0.33 |
| **Sesquiterpeneoxydes** | | | **1.04** |
| 35. | Spathulenol | 1579 | 0.61 |
| 36. | Caryophyllene oxide | 1584 | 0.43 |
| **Total identified components (%)** | | | **96.44** |

*RI - retention index relative to the $C_9$–$C_{25}$ n-alkanes; *the closest isomer; exact isomer not identified*

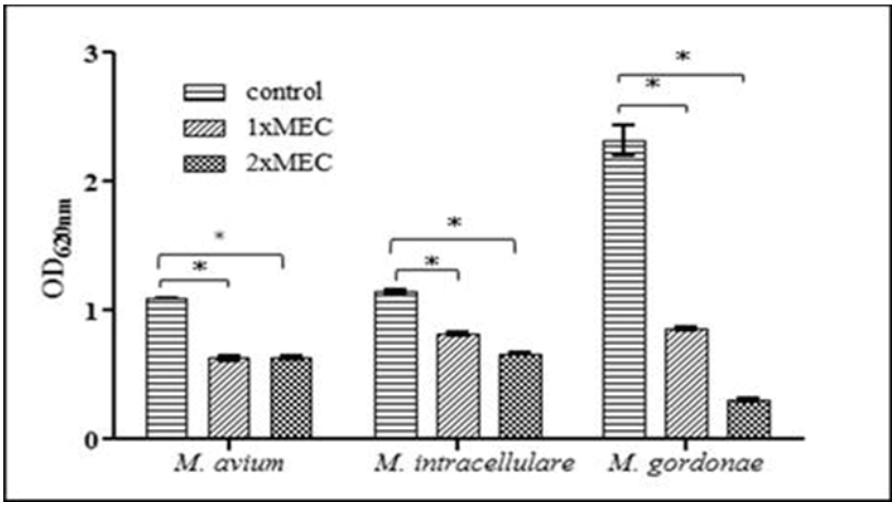


**Figure 2** *Bacteriolytic effects of the juniper EO in sterile tap water after 24 h measured with absorbance at 620 nm*
*Control - unexposed to juniper EO; EO - essential oil; MEC - minimum effective concentration*

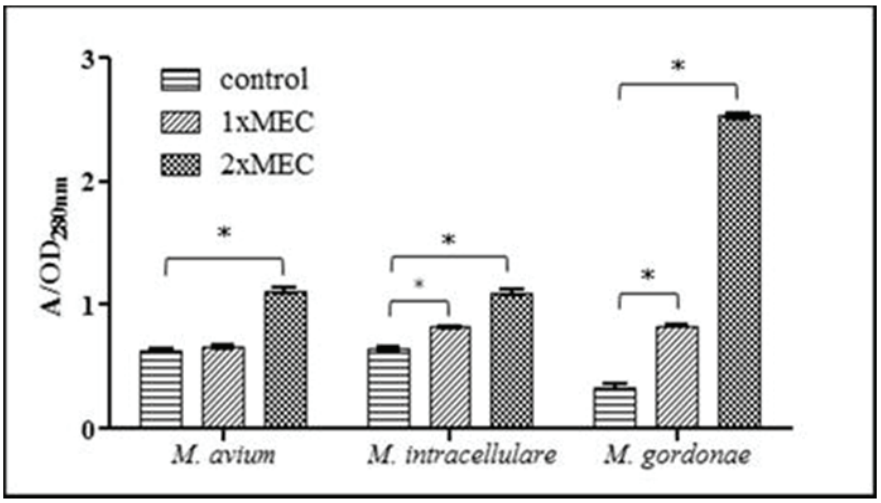


**Figure 3** *Protein leakage after 24 h of treatment with the juniper EO measured with absorbance at 280 nm*
*Control - unexposed to juniper EO; EO - essential oil; MEC - minimum effective concentration*

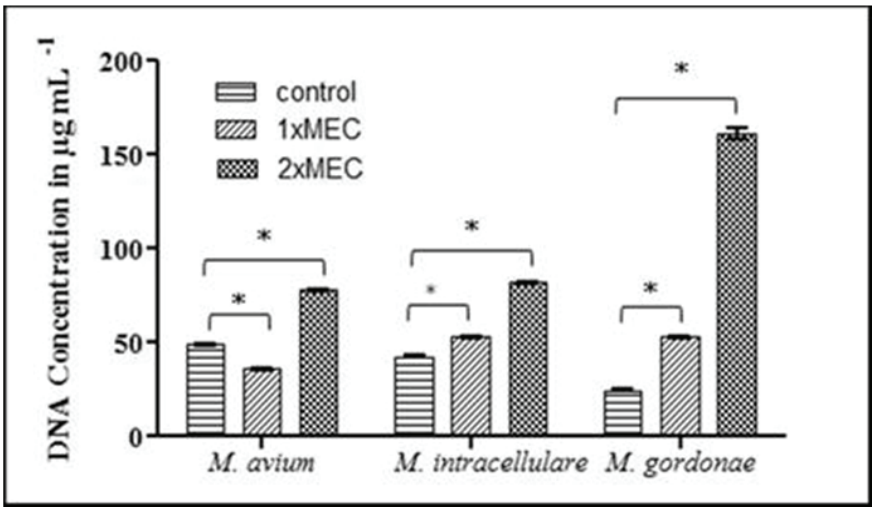


**Figure 4** *DNA leakage after 24 h of treatment with the juniper EO, measured with absorbance at 260 nm*
*Control - unexposed to juniper EO; EO - essential oil; MEC - minimum effective concentration*

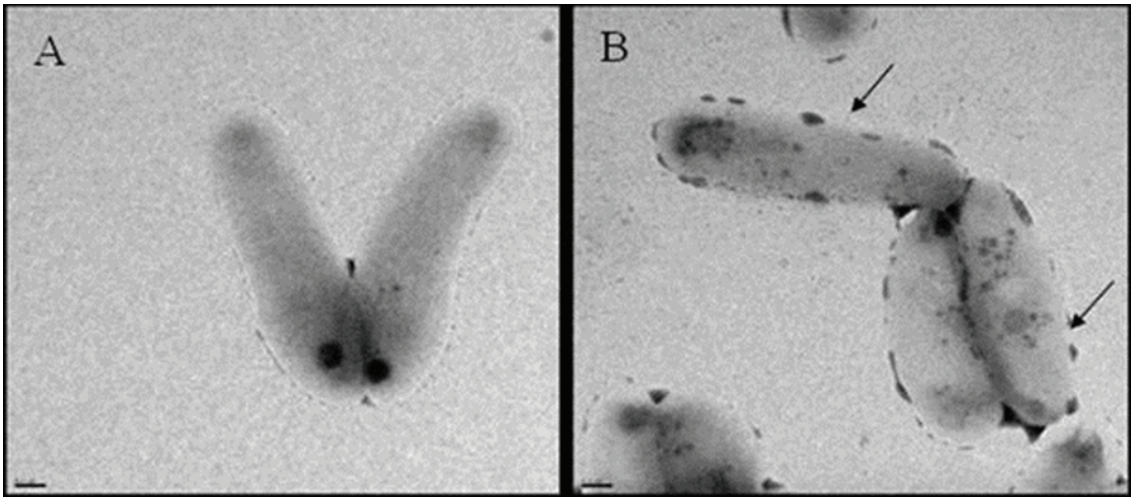


**Figure 5** *Morphological comparison of (A) control* M. avium *cell and (B) M. avium cell treated with the juniper EO at double the MEC for 24 h*
*Control - unexposed to juniper EO; EO - essential oil; MEC - minimum effective concentration*

that EOs penetrate the wall and wreak havoc in the cell. Pinenes have very low water solubility but relatively high antimicrobial activities (37, 39). Such effects of the monoterpenes menthol, thymol, and linalyl acetate have already been described against *Staphylococcus aureus* and *E. coli* (37). Interestingly, even though our juniper EO was dominated by α-pinene, it exhibited three-times stronger inhibition than α-pinene alone (Table 1). This may point to a synergism with other main components in the oil.

Our results have also pointed out differences in MBCs and MECs of the white cedar, laurel, lavandin, and sage EOs when we changed the culture medium from broth to water (Table 1). The reason, we believe, is that, being a medium rich in nutrients, 7H9 promotes NTM division, which renders them much more sensitive to the effects of EOs, whereas nutrient-poor water forces the mycobacteria to adapt and renders them more resistant. However, the change of the medium did not affect the efficiency of the juniper EO, which singles it out as a promising natural and safe way to control mycobacteria in fresh water as source of human infection (7). It could be used as a complementary or alternative disinfectant of hot water systems like baths, swimming pools, spa pools, hot tubs, or even foot baths/ whirlpools.

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**Antimikobakterijski potencijal eteričnog ulja plodova borovice (*Juniperus communis*) u pitkoj vodi**

Ispitali smo aktivnost eteričnih ulja borovice [*Juniperus communis* (*J. communis*)], smilja (*Helichrysum italicum)*, kadulje (*Salvia officinalis)*, lavandina (*Lavandula hybrida)*, lovora (*Laurus nobilis)* i tuje (*Thuja occidentalis)* prema sojevima *Mycobacterium avium* ssp. *avium* (*M. avium*), *Mycobacterium intracellulare* (*M. intracellulare*) i *Mycobacterium gordonae* (*M. gordonae*). Za određivanje minimalnih inhibicijskih koncentracija (MIK) i minimalnih baktericidnih koncentracija (MBK) testiranih eteričnih ulja koristili smo se metodom mikrodiluacije u tekućem bujonu (Middlebrook 7H9), a za određivanje minimalne efektivne koncentracije (MEK) umjesto bujona korištena je sterilna voda iz slavine kojoj je dodano 0,05 % Tweena 80. Dvostruka serijska razrjeđenja eteričnih ulja, počevši od 0,1 do 51,2 mg $mL^{-1}$, u tekućem bujonu ili u sterilnoj vodi iz slavine inokulirana su s mikobakterijskom suspenzijom uz dodatak resazurina. Najučinkovitije eterično ulje prema svim ispitivanim sojevima mikobakterija bilo je eterično ulje *J. communis* s MBK / MIK / MEK vrijednošću od 1,6 mg $mL^{-1}$. Metodom plinske kromatografije i masene spektrometrije analizirano je eterično ulje *J. communis*. Udio monoterpena iznosio je 70,54 %, a seskviterpena 25,9 %. Glavni monoterpenski ugljikovodici bili su α-pinen, sabinen i β-pinen. Eterično ulje *J. communis* pokazalo je značajan inhibicijski učinak na *M. intracellulare* i *M. gordonae* pri MEK i na *M. avium* pri dvostrukim MEK. Elektronskom mikroskopijom kod svih triju sojeva mikobakterija nakon izlaganja djelovanju eteričnog ulja *J. communis* otkrivene su značajne morfološke promjene stanične membrane i citoplazme. Učinak eteričnog ulja *J. communis* na destrukciju stanične membrane mikobakterija potvrđen je značajnim otpuštanjem unutarstaničnog materijala mjerenjem apsorbancije supernatanta pri 260 nm i 280 nm. Zaključno, u našem smo radu razvili novu metodu za ispitivanje antimikrobnog učinka eteričnih ulja ili drugih prirodnih tvari na netuberkulozne mikobakterije koja oponaša uvjete kao u vodenim sustavima. Prirodni proizvodi, osobito eterična ulja, imaju ne samo velik potencijal kao antimikrobni agensi nego i moguću praktičnu primjenu kao alternativni dezinficijensi.

KLJUČNE RIJEČI: *dezinfekcija vode;* Juniperus communis (J. communis); Helichrysum italicum; Lavandula hybrida; Laurus nobilis; *mikrodilucija u vodi; minimalna efektivna koncentracija;* Mycobacterium avium; Mycobacterium gordonae; Mycobacterium intracellulare; *netuberkulozne mikobakterije; pitka voda;* Salvia officinalis; Thuja occidentalis